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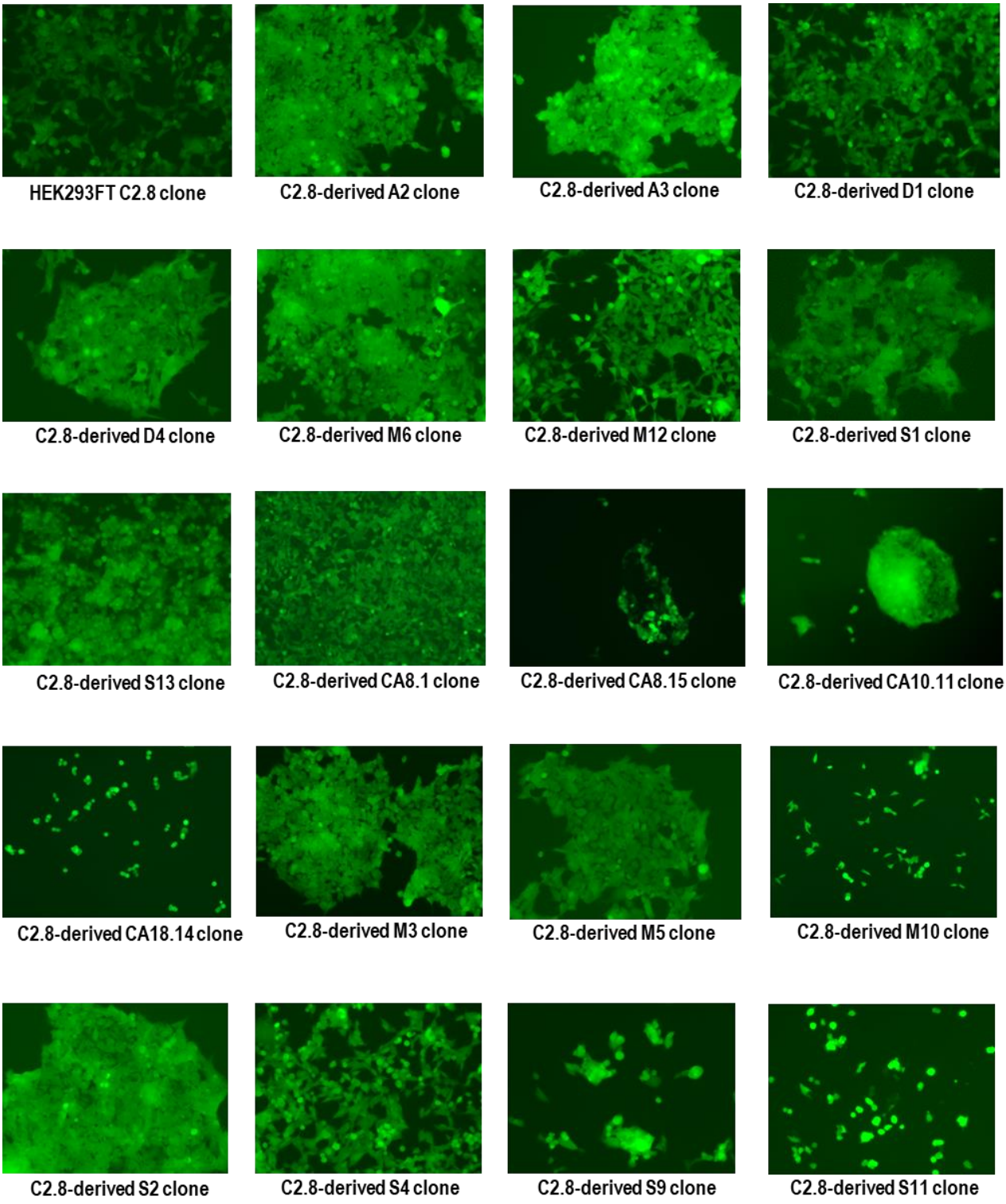
Supplemental information

PAM-flexible dual base editor-mediated random mutagenesis and self-activation strategies to improve CRISPRa potency

Cia-Hin Lau, Siping Huang, Raymond H.W. Lam, and Chung Tin



Supplementary Figure S1. Generation of VP64-mutated clones from HEK293FT C2.8 cells. These GFP-positive cells are expressing CRISPRa (pTK-dSaCas9-VP64-2A-GFP-spA-pU6-sgRNA) that may carry mutated VP64 transgene.

Table S1. Primers used for donor pTK-dSaCas9-VP64-2A-GFP-pU6-sgRNA construction

Primer sequence (5' to 3') (forward, FP; reverse, RP)	Amplicon size (bp)	Description
FP: CACC GACTCGGGCGGGTCGCGCGCCC RP: AAAC GGGCGCGCGACCCGCCCGAGTC	-	for designing sgRNA of CRISPRa to target TK promoter
FP: AAAA TCTAGA AAAA CTCGAG AAATGAGTCTTCGGACCTCGC RP: AAAA ACCGGT TTAAGCGGGTCGCTGCAGGGT	753	PCR TK promoter with added XbaI and XhoI sites PCR TK promoter with added AgeI site
FP: AAAA TCTAGA TGCTTTCTCTGACCAGCATTCTCTCC RP: AAAA CTCGAG CTGTCCCTAGTGGCCCCACTGTG	814	PCR left homology arm from genomic *AAVS1* of HEK293FT
FP: AAAA GCGGCCGC GATTGGTGACAGAAAAGCCCCCATC RP: AAAA GCGGCCGC AGAGCAGAGCCAGGAACCCCTG	821	PCR right homology arm from genomic *AAVS1* of HEK293FT
FP: GCATATACGATACAAGGCTGTTAGAGAG	-	for sequencing pU6-sgRNA

Table S2. Primers used for knock-in of donor pTK-CRISPRa-GFP into genomic *AAVS1* locus

Primer sequence (5' to 3') (forward, FP; reverse, RP)	Amplicon size (bp)	Description
FP: CACC GGGGCCACTAGGGACAGGAT RP: AAAC ATCCTGTCCCTAGTGGCCCC	-	for designing sgRNA of SpCas9 to target genomic *AAVS1* locus
FP: CGCGATCACATGGTCCTGCT RP: CCAAGCAGTCACCCCACAGTTG	1.5k	for verifying targeted integration of pTK-CRISPRa into genomic *AAVS1* locus
FP: CTGAACGCCCATCTGGACATCAC RP: CGCTCCTGGACGTAGCCTTCG	1076	PCR dSaCas9-VP64-2A-GFP
FP: AAATGAGTCTTCGGACCTCGC RP: GTAGCCCACGCTGGTGATGC	867	PCR pTK-dSaCas9
FP: GTAGCCCACGCTGGTGATGC	-	for sequencing HL(AAVS1) and pTK
FP: GACAATCGCCTCCAAGACCCAG	-	for sequencing VP64-2A-GFP
FP: GCATATACGATACAAGGCTGTTAGAGAG	-	for sequencing pU6-sgRNA

Table S3. Primers used for VP64 mutagenesis

Primer sequence (5' to 3') (forward, FP; reverse, RP)	Amplicon size (bp)	Description
FP: CACC GTCAAGGTCAAAATCATCGA RP: AAAC TCGATGATTTTGACCTTGAC	-	for designing sgRNA-1 of base editors to target VP64
FP: CACC GTCGAGGTCAAAGTCATCAA RP: AAAC TTGATGACTTTGACCTCGAC	-	for designing sgRNA-2 of base editors to target VP64
FP: CACC GACTTTGACCTCGACATGCT RP: AAAC AGCATGTCGAGGTCAAAGTC	-	for designing sgRNA-3 of base editors to target VP64
FP: CACC GTCCAGGTCGAAATCATCAA RP: AAAC TTGATGATTTCGACCTGGAC	-	for designing sgRNA-4 of base editors to target VP64
FP: CACC GGACGGGCTGACGCATTGGA RP: AAAC TCCAATGCGTCAGCCCGTCC	-	for designing sgRNA-5 of base editors to target VP64
FP: CACC GCTGACGCATTGGACGATTT RP: AAAC AAATCGTCCAATGCGTCAGC	-	for designing sgRNA-6 of base editors to target VP64
FP: CACC GATCTGGATATGCTGGGAAG RP: AAAC CTTCCCAGCATATCCAGATC	-	for designing sgRNA-7 of base editors to target VP64
FP: CACC GACCTTGACATGCTTGGTTC RP: AAAC GAACCAAGCATGTCAAGGTC	-	for designing sgRNA-8 of base editors to target VP64
FP: CACC GCCCTTGATGACTTTGACCT RP: AAAC AGGTCAAAGTCATCAAGGGC	-	for designing sgRNA-9 of base editors to target VP64
FP: CACC GACTTTGACCTCGACATGCT RP: AAAC AGCATGTCGAGGTCAAAGTC	-	for designing sgRNA-10 of base editors to target VP64
FP: CACC GACCTCGACATGCTCGGCAG RP: AAAC CTGCCGAGCATGTCGAGGTC	-	for designing sgRNA-11 of base editors to target VP64
FP: CACC GCCCTTGATGATTTCGACCT RP: AAAC AGGTCGAAATCATCAAGGGC	-	for designing sgRNA-12 of base editors to target VP64
FP: CACC GTTAATCAGCATGTCCAGGT RP: AAAC ACCTGGACATGCTGATTAAC	-	for designing sgRNA-13 of base editors to target VP64
FP: CACC GTCCAGGTCGAAATCATCAA RP: AAAC TTGATGATTTCGACCTGGAC	-	for designing sgRNA-14 of base editors to target VP64
FP: CACC GAAATCATCAAGGGCGTCAC RP: AAAC GTGACGCCCTTGATGATTTC	-	for designing sgRNA-15 of base editors to target VP64
FP: CACC GTCGAGGTCAAAGTCATCAA RP: AAAC TTGATGACTTTGACCTCGAC	-	for designing sgRNA-16 of base editors to target VP64
FP: CACC GTCAAGGTCAAAATCATCGA RP: AAAC TCGATGATTTTGACCTTGAC	-	for designing sgRNA-17 of base editors to target VP64
FP: CACC GTCATCAAGGGCATCCGAAC RP: AAAC GTTCGGATGCCCTTGATGAC	-	for designing sgRNA-18 of base editors to target VP64
FP: CACC GGCATCCGAACCAAGCATGT RP: AAAC ACATGCTTGGTTCGGATGCC	-	for designing sgRNA-19 of base editors to target VP64
FP: CACC GAACCAAGCATGTCAAGGTC RP: AAAC GACCTTGACATGCTTGGTTC	-	for designing sgRNA-20 of base editors to target VP64
FP: CACC GTCAAAATCATCGAGGGCGT RP: AAAC ACGCCCTCGATGATTTTGAC	-	for designing sgRNA-21 of base editors to target VP64
FP: CACC GTCACTTCCCAGCATATCCA RP: AAAC TGGATATGCTGGGAAGTGAC	-	for designing sgRNA-22 of base editors to target VP64
FP: CTGAACGCCCATCTGGACATCAC RP: CGCTCCTGGACGTAGCCTTCG	1076	PCR VP64 from genomic DNA of CRISPRa-inserted cells for sequencing
FP: GACATCACCTACCGCGAGTACCTG	-	for sequencing VP64
RP: CGCTCCTGGACGTAGCCTTCG	-	for sequencing VP64

Table S4. Primers used for pAAV-CMV-dSaCas9-ΔVP64 construction in luciferase reporter experiments

Primer sequence (5' to 3') (forward, FP; reverse, RP)	Amplicon size (bp)	Description
FP: AAAA TCTAGA TCCGCGTTACATAACTTACGG RP: AAAA ACCGGT AGCTCTGCTTATATAGACCTCCCA	508	PCR CMV with added XbaI site PCR CMV with added AgeI site
FP: CACC GACTCGGGCGGGTCGCGCGCCC RP: AAAC GGGCGCGCGACCCGCCCGAGTC	-	for designing sgRNA-1 of CRISPRa to target TK promoter
FP: CACC GCTCCTGCAGTCCCTCGCGCCT RP: AAAC AGGCGCGAGGGACTGCAGGAGC	-	for designing sgRNA-2 of CRISPRa to target TK promoter
FP: CACC GCGAGGGACTGCAGGAGCTTCA RP: AAAC TGAAGCTCCTGCAGTCCCTCGC	-	for designing sgRNA-3 of CRISPRa to target TK promoter
FP: CACC GCAAACGCGAGCAACGGGCCAC RP: AAAC GTGGCCCGTTGCTCGCGTTTGC	-	for designing sgRNA-4 of CRISPRa to target TK promoter
FP: CACC GTTCAATTACAGCTCTTAAGGC RP: AAAC GCCTTAAGAGCTGTAATTGAAC	-	for designing sgRNA-5 of CRISPRa to target TK promoter
FP: CACC GCCGCGGCGGCGACGGGCTCG RP: AAAC CGAGCCCGTCGCCGCCGCGGC	-	for designing sgRNA-6 of CRISPRa to target TK promoter
FP: CACC GGAGACCTTCTGCGGGACGAG RP: AAAC CTCGTCCCGCAGAAGGTCTCC	-	for designing sgRNA-7 of CRISPRa to target TK promoter
FP: CACC GCCCCGACTGCATCTGCGTGT RP: AAAC ACACGCAGATGCAGTCGGGGC	-	for designing sgRNA-8 of CRISPRa to target TK promoter
FP: CACC GGCGCGCGACCCGCCCGAGT RP: AAAC ACTCGGGCGGGTCGCGCGCC	-	for designing sgRNA of CRISPR/Cas9 to knockout CRISPRa activity
FP: AAAA GGATCC GGACGGGCTGACGCATTGG RP: AAAA GAATTC TTA GTTAATCAGCATGTCCAGGTCG	168	PCR VP64 with added BamHI site PCR VP64 with added EcoRI site and stop codon (only for PCR D1, M12, S13, D4, CA8.1, CA8.15, CA10.11, and CA18.14 of VP64 mutants)
FP: AAAA GGATCC GGACGGGCTGACGCATTGG RP: AAAA GAATTC TTA GTTAATCAGCATGTCCGGGTCG	90 or 129	PCR VP64 with added BamHI site PCR VP64 with added EcoRI site and stop codon (only for PCR M3 and S11 of VP64 mutants)
FP: AAAA GGATCC GGACGGGCTGACGCATTGG RP: AAAA GAATTC TTA GTTAATCAGCATGTTTAGGTCG	159 or 156	PCR VP64 with added BamHI site PCR VP64 with added EcoRI site and stop codon (only for PCR S4 and S9 of VP64 mutants)
FP: AAAA GGATCC GGACGGGCTGACGCATTGG RP: AAAA GAATTC TTA GTTAATCAGCATGTCTAGGTTG	168	PCR VP64 with added BamHI site PCR VP64 with added EcoRI site and stop codon (only for PCR S1 of VP64 mutant)
FP: AAAA GGATCC GGACGGGCTGACGCATTGG RP: AAAA GAATTC TTA GTTAATCAGCATGTGCGGGTCG	134	PCR VP64 with added BamHI site PCR VP64 with added EcoRI site and stop codon (only for PCR M5 of VP64 mutant)
FP: AAAA GGATCC GGACGGGCTGACGCATTGG RP: AAAA GAATTC TTA GTTAATCAGCATGTCGAGGGCGTC	75	PCR VP64 with added BamHI site PCR VP64 with added EcoRI site and stop codon (only for PCR S2 of VP64 mutant)
FP: AAAA GGATCC GGACGGGCTGACGCATTGG RP: AAAA GAATTC TTA GTTAATCAGCATGTTCAGGTCG	168	PCR VP64 with added BamHI site PCR VP64 with added EcoRI site and stop codon (only for PCR A2 of VP64 mutant)
FP: AAAA GGATCC GGACGGGCTGACGCATTGG RP: AAAA GAATTC TTA GTTAATCAGCATGTCCGGGTGG	168	PCR VP64 with added BamHI site PCR VP64 with added EcoRI site and stop codon (only for PCR M6 of VP64 mutant)
FP: AAAA GGATCC GGACGGGCTGACGCATTGG RP: AAAA GAATTC TTA GTTAATCAGCATGTGCAGGTCG	168	PCR VP64 with added BamHI site PCR VP64 with added EcoRI site and stop codon (only for PCR A3 of VP64 mutant)
FP: GACATCACCTACCGCGAGTACCTG	-	for sequencing ΔVP64 in pAAV-CMV-dSaCas9-ΔVP64

RP: CAGGGCGCGTACTATGGTTGC	-	for sequencing ΔVP64 and pU6-sgRNA in pAAV-CMV-dSaCas9-ΔVP64

Table S5. Primers used for pAAV-CMV-dSaCas9-ΔVP64 construction in endogenous gene modulation experiments

Primer sequence (5' to 3') (forward, FP; reverse, RP)	Amplicon size (bp)	Description
FP: AAAA TCTAGA TCCGCGTTACATAACTTACGG RP: AAAA ACCGGT AGCTCTGCTTATATAGACCTCCCA	508	PCR CMV with added XbaI site PCR CMV with added AgeI site
FP: AAAA GGATCC GGACGGGCTGACGCATTGG RP: AAAA GAATTC TTA GTTAATCAGCATGTTCAGGTCG	168	PCR VP64 with added BamHI site PCR VP64 with added EcoRI site and stop codon (only for PCR A2 of VP64 mutant)
FP: AAAA GGATCC GGACGGGCTGACGCATTGG RP: AAAA GAATTC TTA GTTAATCAGCATGTTTAGGTCG	159	PCR VP64 with added BamHI site PCR VP64 with added EcoRI site and stop codon (only for PCR S4 of VP64 mutants)
FP: AAAA GGATCC GGACGGGCTGACGCATTGG RP: AAAA GAATTC TTA GTTAATCAGCATGTCCAGGTCG	168	PCR VP64 with added BamHI site PCR VP64 with added EcoRI site and stop codon (only for PCR CA10.11 of VP64 mutants)
FP: CACC GGGGGCAGGGGCGCCGAGGGCG RP: AAAC CGCCCTCGGCGCCCCTGCCCCC	-	for designing sgRNA site-10 of CRISPRa to target human *KLOTHO* promoter
FP: CACC GGCGCGCTCCGCTAGGGCCCGG RP: AAAC CCGGGCCCTAGCGGAGCGCGCC	-	for designing sgRNA site-11 of CRISPRa to target human *KLOTHO* promoter
FP: CACC GGGAACTTCGCCGTGCGCTGAA RP: AAAC TTCAGCGCACGGCGAAGTTCCC	-	for designing sgRNA site-12 of CRISPRa to target human *KLOTHO* promoter
FP: CACC GGCGGCCGCCCCAATTTCCCGC RP: AAAC GCGGGAAATTGGGGCGGCCGCC	-	for designing sgRNA site-9 of CRISPRa to target human *SIRT6* promoter
FP: CACC GCATGCGCCTTGCCGTGGGAGG RP: AAAC CCTCCCACGGCAAGGCGCATGC	-	for designing sgRNA site-10 of CRISPRa to target human *SIRT6* promoter
FP: CACC GGACAGCAGGGACCCCAGCCT RP: AAAC AGGCTGGGGTCCCTGCTGTCC	-	for designing sgRNA site-11 of CRISPRa to target human *SIRT6* promoter
FP: CACC GCGTCAGCCCCGGCGCGGGTG RP: AAAC CACCCGCGCCGGGGCTGACGC		for designing sgRNA site-1 of CRISPRa to target human *NFE2L2* promoter
FP: CACC GGGGCGGGAAGGGACTGCCAGC RP: AAAC GCTGGCAGTCCCTTCCCGCCCC		for designing sgRNA site-2 of CRISPRa to target human *NFE2L2* promoter
FP: CACC GGGCAGTTGGCAGTGGCACGGT RP: AAAC ACCGTGCCACTGCCAACTGCCC		for designing sgRNA site-3 of CRISPRa to target human *NFE2L2* promoter
FP: GCATATACGATACAAGGCTGTTAGAGAG	-	for sequencing pU6-sgRNA

Table S6. Primers used for AAV titters

Primer sequence (5' to 3') (forward, FP; reverse, RP)	Amplicon size (bp)	Description
6-FAM-CACCACGCCGAGGACGCCCTGA-ZEN/Iowa	-	probe specific to dSaCas9
FP: TCCATCAATGGCGGCTTCA RP: GGCCTTGTCCAGTTTCTTCCA	150	quantify the copies number of dSaCas9 DNA

Table S7. Primers used for qRT-PCR and TaqMan qPCR

Primer sequence (5' to 3') (forward, FP; reverse, RP)	Amplicon size (bp)	Description
FP: ACCATCTTCCAGGAGCGAGA RP: TGGCATGGACTGTGGTCATG	319	qPCR mRNA expression level of human *GAPDH*
FP: ACCAAGAGAGATGATGCCAAATA RP: CCACTCGAAACCATCCATGA	132	qPCR mRNA expression level of human *KLOTHO*
FP: CCCACGGAGTCTGGACCAT RP: CTCTGCCAGTTTGTCCCTG	194	qPCR mRNA expression level of human *SIRT6*
FP: CACATCCAGTCAGAAACCAGTGG RP: GGAATGTCTGCGCCAAAAGCTG	112	qPCR mRNA expression level of human *NFE2L2*